ELECTRONIC SOMATIC CELL COUNT

Bentley Somacount 150/300/500

(Unless otherwise stated all tolerances ±5%)

1. Laboratory requirements (see CP, item 33 & 34) _____

   a. Un-preserved samples may be run up to 72 hours after initial collection _____

   b. Samples may be run up to 7 days after initial collection if preserved with 0.02% 2-bromo-2-nitropropane-1,3-diol (Bronopol™) or 0.05% potassium dichromate ($K_2Cr_2O_7$) _____

   c. Comparative test with DMSCC _____

      1. Performed by each analyst performing ESCC test _____

      2. Test 4 samples (100K-200K, 300K-500K, 600K-800K and 900K-1.2M) in triplicate for both DMSCC (three separate smears each) and ESCC (three separate sub-samples each, do not read same sample three times) _____

      3. Results must be shown to be acceptable prior to official testing by analyst performing comparison, i.e. analyst is not certified until found acceptable. **(co-requisite for certification)** _____

      4. Copy of comparison and results in QC record (or easily accessible file in laboratory) _____

   d. Analysts certified in DMSCC _____

APPARATUS

2. Cultural procedures, items 1-5 _____

3. Electronic Somatic Cell Counter _____

   a. Bentley Somacount™ 150 _____ _____

   b. Bentley Somacount™ 300 _____ _____

   c. Bentley Somacount™ 500 _____ _____

4. Water bath _____

   a. Circulating and thermostatically controlled to 37-42C _____

REAGENTS

5. Stock Dye/Buffer Solution _____

   a. Dissolve 80g of tripotassium citrate monohydrate, ($K_3C_6H_5O_7,H_2O$), 3.0g of citric acid monohydrate ($C_6H_8O_7,H_2O$), and 0.25g (1 tablet) of ethidium bromide ($C_{21}H_{20}BrN_3$) in 750 mL of deionized (DI) or MS water. Heat to 40-60C and stir until totally dissolved (**Caution:** Ethidium bromide is **TOXIC**. When handling, avoid contact with skin and do not breathe dust.) _____

   b. Add 10 mL of neutral detergent, Triton X-100, and stir until totally dissolved. Adjust volume to 1 Liter with DI or MS water _____

   c. Store refrigerated (0-4.4C) in airtight, light-proof container for no longer than 90 days _____

   d. Container labeled with date prepared and expiration date _____

      Date prep. _____ Exp. Date _____ _____

WORKING SOLUTIONS

6. Dye/Buffer Solution _____

   a. Dilute 1 part of Stock Dye/Buffer solution with 9 parts of DI or MS water _____

   b. Protect from light and use within 21 days _____

   c. Date prep. _____ Exp. Date _____ _____

7. Rinse Solution _____

   a. Add 20 mL of alkaline detergent, RBS-35, per liter of DI or MS water and mix _____

   b. Use within 7 days _____

   c. Date prep. _____ Exp. Date _____ _____

8. Optionally use manufacturer's reagent kits and instructions _____

9. All dye/buffer and rinsing solutions labeled with date prepared and expiration date _____

START UP

10. Cell Counter _____

    a. Check that the amount of dye/buffer solution (item 6) and rinse solution (item 7) in the supply containers is of sufficient volume for the number of samples to be run _____

    b. Solutions not to be used beyond expiration date(s) _____

    c. Turn on computer and instrument, wait 20 minutes before proceeding _____

    d. Laser power > 0.25 mW _____

    e. |PMT voltage| > 10 mV _____

    f. Coil temperature between 67-73C _____

    g. Run DI OR MS water at least 3 times; reading must be zero (0) on every test _____

    h. **IF ANY PARAMETERS ARE OUT OF TOLERANCE, CORRECT BEFORE PROCEEDING** _____

    i. Records maintained on all parameters each time instrument is used _____

11. Milk Standards _____

    a. Commercially prepared: _____
    Lot# _____ Date Rcd. _____ _____

        1. Four standards in ranges 100K-200K, 300K-500K, 600K-800K and 900K-1.2M _____

        2. Do DMSCC in triplicate on each standard in set and average counts, records maintained _____

        3. DMSCC check performed in rotation by all certified analysts _____

        4. Standards used within one week _____

    b. Certified provider: _____

    Lot# _____ Exp. Date _____ Date Rcd. _____ _____

        1. Four standards in ranges 100K-200K, 300K-500K, 600K-800K and 900K-1.2M _____

        2. Maintain copies of all provided DMSCC values _____

        3. Measure and maintain records of temperature (0-7.2C) of standards as received _____

4. Maintain copies of all correspondence regarding problems _____

5. Standards used by manufacturer's expiration date _____

c. Laboratory prepared (weekly) _____

1. Prepare from raw milk > 18 hours old preserved with 0.05% potassium dichromate ($K_2Cr_2O_7$) _____

2. Or, preserved with 0.02% 2-brono-2-nitropropane-1,3-diol (Bronopol™) _____

3. Standards <u>cannot</u> be preserved with formalin _____

4. Prepare 4 standards in ranges 100K-200K, 300K-500K, 600K-800K and 900K-1.2M, use within one week
Date prep. _____ Exp. Date _____ _____

5. Do DMSCC in triplicate on each standard prepared and average counts, records maintained _____

6. DMSCC check performed in rotation by all certified analysts _____

d. Hourly Control Sample (instrument drift check) _____

1. Use one of the standards (items 11a or b) in the 500-800K range, run in triplicate and determine average _____

2. Optionally, prepare sufficient control/sample 500-800K range, run in triplicate and determine average _____

PROCEDURE

12. Testing Standards (each time instrument used) _____

a. Heat standards to 37-42C (using a temperature control) and read within 30 minutes of reaching temperature, used once and then discarded, i.e., do not re-use _____

b. Mix by inverting at least 2x, test standards within 3 minutes _____

c. Run the standards in triplicate and average the counts for each level, records maintained _____

d. Each standard's average must be within 10% of the DMSCC (item 11) for that level, except within 15% for 100-200K standard, records maintained _____

e. Repeatability - a standard in the 300K to 800K range must have a coefficient of variation (C_v) of 5% or less on 10 replicates **(Refer to Operating Manual)**, records maintained _____

f. **THESE PARAMETERS MUST BE ACHIEVED BEFORE PROCEEDING** _____

13. Testing samples _____

a. Heat samples to 37-42C (using a temperature control) and read within 30 minutes of reaching temperature; samples must not be re-used and must be discarded after use _____

b. Mix by inverting at least 2x, test samples within 3 minutes _____

c. Samples must be tested within 10 minutes after being removed from waterbath _____

d. Samples must not be reused and must be discarded after use _____

e. Record number of cells counted for each sample _____

14. With continuous operation:

a. Run a standard or optionally a control/sample (item 11d) in the 500K to 800K range hourly, must be within 5% of the original established instrument average value (optionally, within 10% of original DMSCC average) _____

b. Run control 3x _____

c. Run zero control (item 10g) hourly _____

d. Maintain records _____

15. Routine maintenance _____

a. Perform as described in operating manual _____

b. Maintain records _____

REPORTS

16. Computing and Reporting of Counts _____

a. Count obtained x 1000 is the cell count/mL milk _____

b. In reporting electronic somatic cell counts (ESCC/mL), record only first two left hand digits, raising second digit to next higher number when third digit is six or more _____

c. Report the two left hand digits (rounded) _____

1. If the third digit is 5 the second digit is rounded by the following rule _____

a. When second digit is odd round up, raising the second digit by 1 (odd up, 235 to 240) _____

b. When second digit is even round down, delete the 5 and report the second digit as is (even down, 225 to 220) _____

d. If count on instrument is < 100 report count as < 100,000 ESCC/mL _____